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A SPECTROPHOTOMETRICAL METHOD FOR DETERMINATION OF DISSOLVED PROTEINS IN WATER OR WASTEWATER

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NOMENCLATURE

Br	bromine
$CuSO_4$	copper sulfate
HCl	hydrochloric acid
H_3PO_4	phosphoric acid
KNOX	Knox Gelatin Inc.
Li_2SO_4	lithium sulfate
NaOH	sodium hydroxide, caustic soda
Na_2CO_3	sodium carbonate
$Na_2C_4H_4O_6\text{-}2H_2O$	sodium tartrate
$Na_2MoO_4\text{-}2H_2O$	sodium molybdate
$Na_2WO_4\text{-}2H_2O$	sodium tungstate

A SPECTROPHOTOMETRICAL METHOD FOR DETERMINATION OF DISSOLVED PROTEINS IN WATER OR WASTEWATER

1. INTRODUCTION

Organic waste products of plants and animals are categorized into three groups: (a) lipids, (b) carbohydrates and (c) proteins. While lipids and carbohydrates contain only carbon, hydrogen, and oxygen, proteins contain additional sulfur nitrogen and occasionally phosphorous.

According to the University of Massachusetts, proteins are highly organized polymers of amino acids with peptide linkages. The number, type and arrangement of the amino acids determine the differences between protein molecules. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.*, tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique functional properties, *i.e.*, their ability to

provide desirable appearance, texture or stability.

The amount of protein in certain waste waters is an important parameter to consider when evaluating the quality of specific waters and in the determination of appropriate treatment processes. While various methods have been suggested for this analysis, it would be desirable to effect a method suitable for rapid determination of dissolved proteins in water and wastewater either in a laboratory or in the field.

2. LITERATURE REVIEW

The reported protein content of foods and any organic matter depends on the analytical method used for determination, making a direct comparison between studies difficult. (1-30). Maehre et. al. have examined and compared many protein analytical methods (1-27), and have concluded that (a) most analytical methods overestimated the protein contents; (b) the inaccuracies were linked to indirect measurements, i.e., nitrogen determination and subsequent conversion to protein, or interference from other chemical substances; and (c) amino acid analysis is the only protein analysis method where interfering substances do not affect the results and this method should be the preferred for food protein determination. (28).

Another overview of protein assays methods has been done by Protein Biology Resource Library (29). The Library states that (a) The simplest and most direct assay method for proteins in solution is to measure the absorbance at 280 nm (UV range). Amino acids containing aromatic side chains (i.e., tyrosine, tryptophan and phenylalanine) exhibit strong UV-light absorption. Consequently, proteins and peptides absorb UV-light in proportion to their aromatic amino acid content and

total concentration. (b) Another method, traditionally used in amino acid analysis by HPLC, is to label all primary amines (i.e., N-terminus and side-chain of lysine residues) with a colored or fluorescent dye such as ninhydrin or o-phthalaldehyde (OPA). Direct UV-light absorbance and HPLC-reagent approaches have particular disadvantages that make them impractical for use with typical protein samples in proteomics workflows. (c) Several colorimetric and fluorescent, reagent-based protein assay techniques have been developed that are used by nearly every laboratory involved in protein research. Protein is added to the reagent, producing a color change or increased fluorescence in proportion to the amount added. Protein concentration is determined by reference to a standard curve consisting of known concentrations of a purified reference protein. (29)

These colorimetric and fluorescent, reagent-based protein assay techniques can be divided into two groups based on the type of chemistry involved : (a) Biuret method or a modified Lowry method: Protein-copper chelation and secondary detection of the reduced copper; and Bradford method: protein-dye binding and direct detection of the color change or increase in fluorescence associated with the bound dye. (29)

The University of Massachusetts, USA, (30) has described and discussed the principles, methods, advantages and disadvantages of the following common protein analytical methods: (a) Kjeldahl method; (b) enhanced Duas method; (c) UV-visible spectroscopy methods; and (d) some other instrumental methods.

Among the UV-visible spectroscopy methods, the University of Massachusetts (30) has discussed: (a) Lowry method; (b) Bradford dye-binding method; and (c) turbimetric method.

The above literature sources have reported many methods for determination of proteins in mainly foods. The authors' UV-visible spectroscopy method is a modified Biuret-Lowy protein-copper chelation method specifically developed for determination of dissolved protein in water or wastewater.

3. PRINCIPLE

The new analytical method is a colorimetric determination based on the reaction of dissolved protein, specifically the peptide bonds and the amino acids tryptophan and tyrosine with the suggested reagents. Two reactions are necessary:

(a) The reaction between the protein molecules and copper ions in an alkaline solution and

(b) The reduction of a phosphomolybdic phototungstic reagent by the copper treated protein.

The optical density of the treated dissolved protein is then determined at 400-700 nm range using a spectrophotometer with 1 cm light path or longer. This method may be used in the field after calibration curves are prepared in a laboratory.

4. SAMPLING AND STORAGE

To conduct the dissolved protein analysis in water or wastewater, a sample of only 10 mL is used.

Samples may be collected and placed in 50 mL capped test tubes in which the chemical reactions will take place.

If the analysis is to be done at a later time a 100 mL sample may be collected, capped and stored in a refrigerator. Aliquot 10 mL samples for analysis later.

All sample containers should be sterilized in a microwave (2), or an autoclave.

5. INSTRUMENTATION

A number of methods have been devised to measure protein concentration, which are based on UV-visible spectroscopy. These methods use either the natural ability of proteins to absorb (or scatter) light in the UV-visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb (or scatter) light in this region. The basic principle behind each of these tests is similar. First of all a calibration curve of absorbance (or turbidity) versus protein concentration is prepared using a series of protein solutions of known concentration. The absorbance (or turbidity) of the solution being analyzed is then measured at the same wavelength, and its protein concentration determined from the calibration

curve. The main difference between the tests are the chemical groups which are responsible for the absorption or scattering of radiation, *e.g.*, peptide bonds, aromatic side-groups, basic groups and aggregated proteins. The following are the recommended spectrophotometers for dissolved protein analyses in water or wastewater.

5.1. HACH DR 2, or DR 3900 Spectrophotometer, or Equivalent

Section 9 shows a HACH 3900 Spectrophotometer.

5.2. Baush & Lomb Spectronic 710, or Spectronic 2000, or Equivalent

Section 9 shows a Baush & Lomb Spectronic 2000.

6. REAGENTS

6.1. Carbonate-Tartrate Reagent:

Dissolve 200 grams sodium carbonate Na_2CO_3 and 12 grams sodium tartrate $Na_2C_4H_4O_6 \cdot 2H_2O$ in 750 mL hot distilled water. Cool the solution to 25 degree C, and dilute it to 1 liter.

6.2. Phosphomolybdic-Phosphotungstic Reagent:

Transfer 100 grams sodium tungstate, $Na_2WO_4 \cdot 2H_2O$, and 25 grams sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, together with 700 mL distilled water, to a 2000-mL flat-bottom boiling flask. Add 50 mL 85% H_3PO_4 and 100 mL concentrated HCl acid to the flask. Connect the flask to a reflux condenser and boil gently for 10 hours. Add 150 grams Li_2SO_4, 50 mL distilled water, and a few drops of liquid bromine. Boil the flask without condenser for 15 min. to remove excess bromine under an exhaust hood. Cool to 25°C, dilute to 1 liter, and filter. Store finished reagent, which should have no greenish tint, in a tightly stoppered bottle to protect against any chemical reduction by dust and organic materials.

6.3. Sodium Hydroxide Solution, 2.0 N:.

Dissolve 40 grams sodium hydroxide, NaOH, in 500 mL ammonia-free distilled water. 1 mL = 80 mg NaOH.

6.4. Copper Sulfate Solution:

Dissolve 1.00 gram copper sulfate, $CuSO_4$, in 1 liter distilled water.
1 mL = 1 mg $CuSO_4$
CT, and Wang LK , editors)

6.5. Stock Protein Solution:

Weigh 1.00 gram gelatin or bovine. Dissolve the protein (gelatin or bovine) in distilled water and dilute the solution to l,000 mL. Example: 1.00 gram of pure gelatin corrected to 1.14 gram to compensate for 86% protein in commercial KNOX unflavored gelatin (supplied by Knox Gelatin Inc., Johnstown, NY 12095, U.S.A.). Heat the protein (gelatin or bovine) and distilled water mixture on low heat with slow stirring to dissolve protein (gelatin of bovine) approximately in 20-30 minutes depending on concentration and the type of protein selected as a standard protein.
1 mL = 1 mg active ingredient of protein.

6.6. Standard Protein Solution:

Dilute 10 mL Stock Protein Solution to 1,000 mL with distilled water. 1 mL = 10 micrograms active ingredient.

Dilute 50 mL Stock Protein Solution to 1,000 mL with distilled water. 1 mL = 50 micrograms active ingredient.

7. DEVELOPMENT OE CALIBRATION CURVES USING PROTEIN STANDARDS

7.1. Preparation of Protein Standards

Using the Standard Protein Solution, prepare protein standards at concentrations of 10 mg/L, 100 mg/L, 500 mg/L, 1,000 mg/L, 2,500 mg/L, 5,000 mg/L, 10,000 mg/L, and 30,000 mg/L.

7.2. Preparation of Protein Samples in Test Tubes

Prepare each Protein Sample with 2 mL of each Protein Standard and 8 mL distilled water. Place each Protein Sample in a test tube, and perform the following with each concentration separately. Each Protein Sample (or each 2mL Protein Standard plus 8 mL dilution water) should be equal to 10 mL and should be placed in a test tube separately.

7.3. NaOH Treatment of and Ammonia Removal from Protein Samples.

Add 10 mL of sodium hydroxide solution to each Protein Sample. Cap each test tube with a glass marble and boil it for 5 minutes. Boiling may be eliminated if the sample is known to contain no ammonia.

7.4. Further Protein Sample Treatment with Carbonate Tartrate Reagent and Copper Sulfate Solution

Cool protein samples to room temperature. Add two mL of carbonate tartrate reagent, and two mL of copper sulfate solution to each protein sample. Mix thoroughly and let stand for 15 minutes at room temperature.

7.5. Still Further Protein Sample Treatment with Phosphomolybdic Phosphotungstic Reagent

Add two mL of phosphomolybdic phosphotungstic reagent to the sample and mix immediately.

7.6. Color Development and Sample Filtration

Let color develop for 30 minutes at room temperature. After 30 minutes the test tube contents may be filtered to remove any precipitate through a glass microfiber filter.

7.7. Optical Density Determination Using a Spectrophotometer

Optical density is measured in a spectrophotometer, can be used as a measure of the concentration of substances in a suspension. As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria or other material is present. Optical density measures the amount of attenuation, or intensity lost, when light passes through an optical component. It also tracks attenuation based on the scattering of light, whereas absorbance considers only the absorption of light within the optical component.

Determine optical density at 400-700 nm with a spectrophotometer. For accuracy, optical density should fall between 0.4 and 1.8 if possible (Note: Try different wavelengths and light path lengths. Zero the instrument with pure distilled water).

7.8. Distilled Water Blank Control

Always prepare a reagent blank each time this test is performed. Use distilled water in the reagent blank.

7.9. Calibration Curves Plotting

Plot Absorbance vs. Concentration at different wavelengths to derive the calibration curves. Figures 1 to 3 are the examples. An analytical chemist should prepare his/her own calibration curves using his/her own target protein.

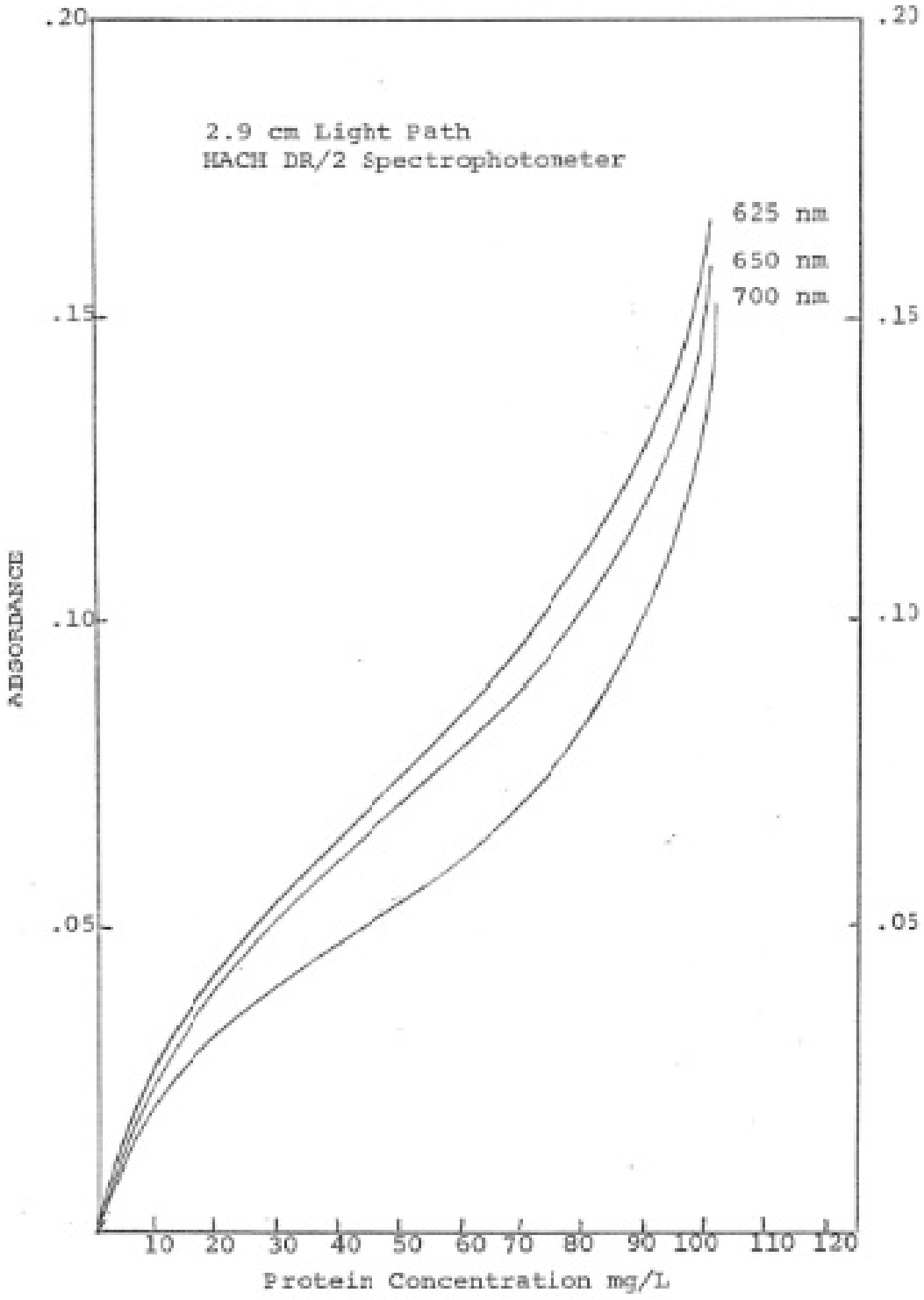

FIGURE 1. Sample Calibration Curve for Dissolved Protein (10-100 mg/L)

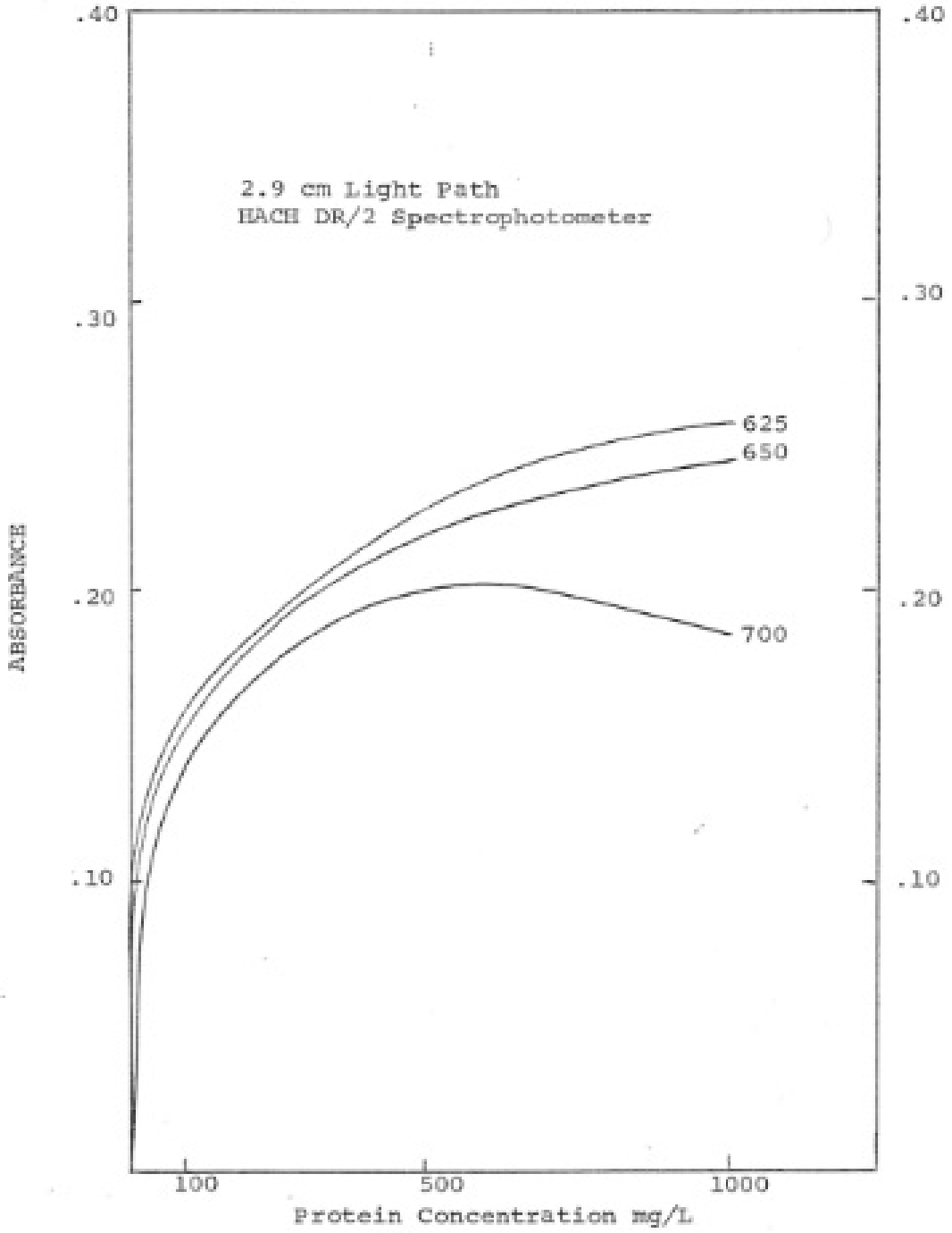

FIGURE 2. Sample Calibration Curve for Dissolved Protein (100-1000 mg/L)

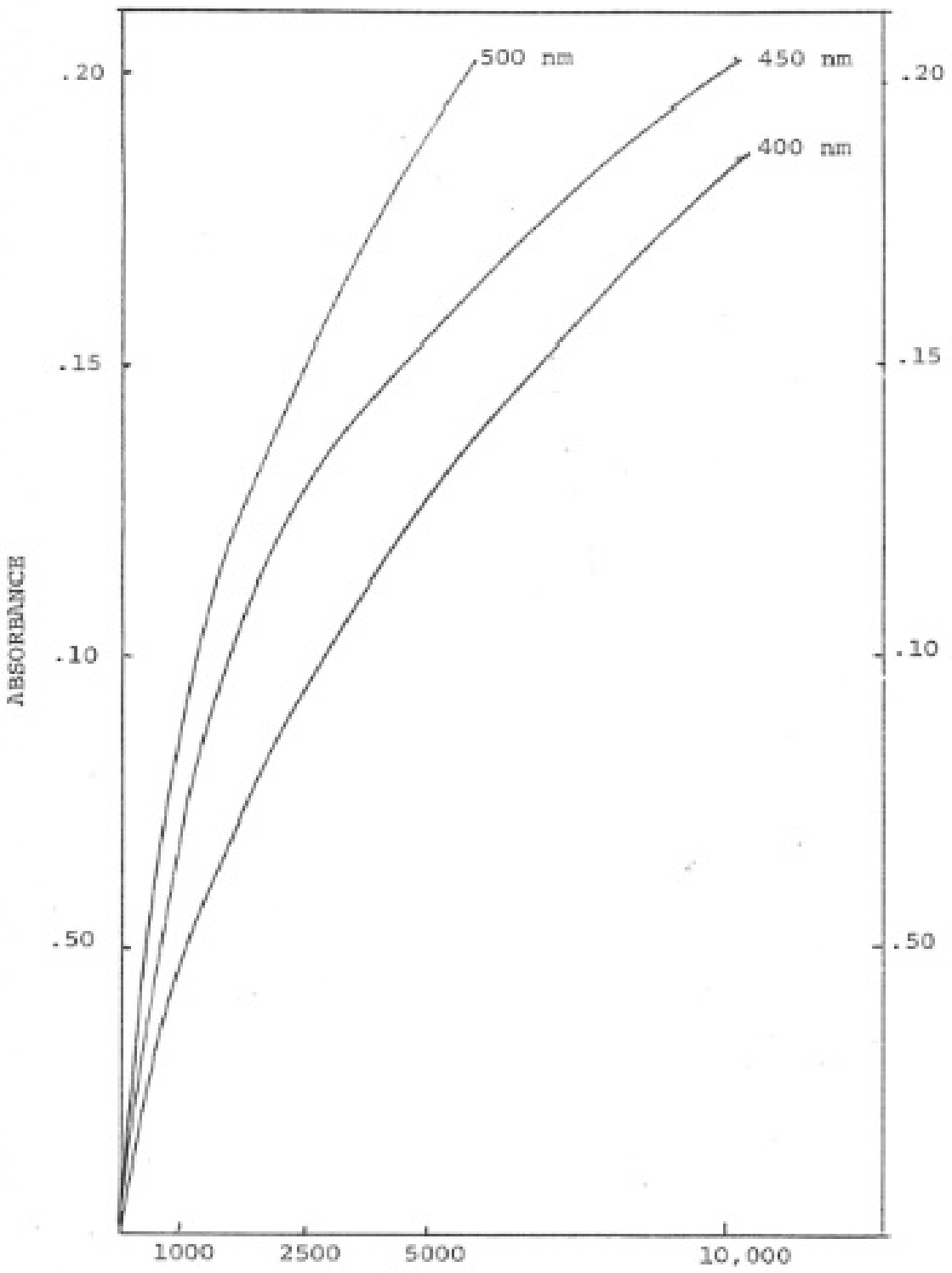

FIGURE 3. Sample Calibration Curve for Dissolved Protein (1000-10,000 mg/L)

8. PROCEDURE FOR WATER OR WASTEWATER SAMPLE ANALYSIS

8.1 Preparation of Water/Wastewater Samples in Test Tubes

The water or wastewater sample (or the sample plus dilution water) should equal 10 mL and is placed in a test tube. If dilution is required, use 2 mL real water or wastewater sample, 8 mL distilled water.

8.2. NaOH Treatment of and Ammonia Removal from Water/Wastewater Samples.

Add 10 mL of sodium hydroxide solution to each sample. Cap each test tube with a glass marble and boil it for 5 minutes. Eliminate boiling step in ammonia free waters.

8. 3. Further Water/Wastewater Sample Treatment with Carbonate Tartrate Reagent and Copper Sulfate Solution

Cool sample and add two mL of carbonate tartrate reagent, and two mL of copper sulfate solution to each test tube. Mix thoroughly, and let stand for 15 minutes at room temperature.

8.4. Still Further Water/Wastewater Sample Treatment with Phosphomolybdic Phosphotungstic Reagent

Add two mL of phosphomolybdic phosphotungstic reagent to each test tube and mix it immediately.

8.5. Color Development and Water/Wastewater Sample Filtration

Let color develop for 30 minutes at room temperature. After 30 minutes the test tube contents may be filtered to remove precipitate through a glass microfiber filter.

8.6. Optical Density Determination Using a Spectrophotometer

Determine optical density at 400-700 nm with a spectrophotometer. For accuracy, optical density should fall between 0.4 and 1.8 if possible (Note: Try different wavelengths and 1ight path lengths.) Zero the instrument with distilled water.

8.7. Distilled Water Blank Control

Always prepare a reagent blank each time this test is performed. Use distilled water in the reagent blank.

8.8. Protein Concentration Determination Using the Calibration Curves

Use the calibration plots from the protein standards (such as Figures 1 to 3) to determine the concentration of protein in the water or wastewater sample. Read the absorbance to determine the protein concentration. If the absorbance from one calibration curve exceeds 1.8 for a sample, select another wavelength or dilute water or wastewater sample as required.

9. PRECISION AND ACCURACY

Precision is the degree to which a set of observations or measurements of the same property, usually obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms.

Accuracy is the degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. USEPA recommends that this term not be used and that precision and bias be used to convey the information usually associated with accuracy.

Protein Standards down to concentrations of 10 mg/L were found to be measurable in the HACH DR 2 Spectrophotometer, or HACH DR 3900 (Figure 4), or Baush & Lomb Spectronic 710, or Spectronic 2000 (Figure 5), or equivalent. Significant color change was noted in samples held over 30 minutes. Therefore the analysis after addition of the second reagent should be completed within 30 minutes or as soon as possible.

Besides, if pure protein samples of the specific protein being tested for are available, these would be ideal for use in calibration curve preparation.

Figure 4. A HACH 3900 Spectrophotometer

Figure 5. A Baush & Lomb Spectronic 2000.

10. GENERAL ANALYTICAL CONDITIONS AND WASTE MINIMIZATION

10.1 General Analytical Conditions

10.1.1 Instrument:

Use HACH DR 2, or DR 3900 Spectrophotometer, or Baush & Lomb Spectronic 710, or Spectronic 2000, or equivalent.

10.1.2 Sample Sizes

Use 250 mL protein standard prepared; 10 mL sample size

10.1.3 Blank

Use distilled water for zeroing the spectrophotometer.

10.1.4 Dilution Factor

Use 1 for “no dilution”, and figure out the rest dilution factor accordingly.

10.1.5. Protein Standard

Use Knox Pure Gelatin containing 85-87% protein (Knox Gelatine Inc., Johnstown, NY 12095, USA), or the analyst’s better choice of pure protein.

10.1.6. Filter Paper

Use 11.0 cm 934.AH Whatman Glass microfiber filters., or equivalent.

10.2. Waste Minimization and Chemical Wastes Neutralization

10.2.1 Waste Minimization

It is suggested by Wang, et. al. (31-32) that every analytical method must consider laboratory waste minimization and hazardous waste management issues. All laboratory wastes generated from the analyses of dissolved protein in water and wastewater must be properly managed and disposed of in accordance with the national, state and local environmental regulations.

A hazardous waste is a material that is subject to special consideration by the USEPA, under 40CFR261. State or local authorities may also designate additional materials as hazardous waste in their areas. The definition given by 40 CFR 261 defines a hazardous waste as a solid waste that is not excluded from regulation and meets one or more of the following criteria: (a) it is a discarded commercial chemical product, off-specification species, container residue, or spill residue of materials specifically listed in 40CFR261.33 (P- and U-codes); (b) it is a waste from a specific source listed in 40CFR261.32 (K-code); (c) it is a waste from a non-specific source listed in 40CFR261.31 (F-code); and/or (d) it displays any of the following characteristics of hazardous wastes: ignitability (such as flash point is below 60 degree C or 140 degree F, it it is classified by DOT as an oxidizer D001), corrosivity (such as the pH of the waste material is less than or equal to 2, or greater than or equal to 12.5, or classified by DOT as D002), reactivity (such as the waste material is unstable, reacts violently with water, may generate toxic gases when mixed with water, or classified by DOT as D003), or toxicity (such as it is classified by DOT ad D004-D043). (34-35)

The most effective ways to laboratory hazardous waste management are: (a) using the smallest amount of chemicals and smallest sample size that will still produce accurate analytical results; (b) choosing chemical analytical methods that use reagents posing less toxic, fewer hazards, or lower discharge of green house gases; (c) using biodegradable detergents nd on-time use laboratory supplies; (d)

separating hazardous waste from non-hazardous wastes in order to minimize the total hazardous waste quantity; (e) clearly labeling all hazardous, toxic, or infectious wastes for handling by the downstream licensed TSDFs (Treatment, Storage, and Disposal Facilities); and (f) getting a permit from the USEPA (US Environmental Protection Agency) and/or state agency for the allowable waste treatment. The most common allowed chemical treatment is elementary neutralization. This applies to wastes that are hazardous only because they are corrosive, or are listed only for that reason.

10.2.2 Chemical Wastes Neutralization

Acidic wastes can be neutralized by adding a base such as calcium hydroxide, potassium hydroxide, or sodium hydroxide. Basic wastes can be neutralized by adding an acid such as sulfuric acid, hydrochloric acid, phosphoric acid, etc. Slowly add the neutralizing chemical while stirring. Monitor this liquid mixture's pH. When it is at or near 7, the waste mixture is neutralized and maybe flushed down the laboratory drain. Many wastes generated from this dissolved protein analysis may be handled in this manner. The readers are referred to the literature (33) for the theory and principles of neutralization reactions.

11. GLOSSARY (30, 34, 35, 36)

Absolute method: a body of procedures and techniques for which measurement is based entirely on physically defined, fundamental quantities.

Accuracy: the degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. USEPA recommends that this term not be used and that precision and bias be used to convey the information usually associated with accuracy.

Acid: any substance capable of giving up a proton; a substance that ionizes in solution to give the positive ion of the solvent; a solution with a pH measurement less than 7.

Acidity: the quantitative capacity of aqueous solutions to react with hydroxyl ions. It is measured by titration with a standard solution of base to a specified end point.

Aliquot: a subsample derived by a divisor that divides a sample into a number of equal parts and leaves no remainder; a subsample resulting from such a division. In analytical chemistry the term aliquot is generally used to define any representative portion of the sample.

Alkalinity: the capacity of water to neutralize acids, a property imparted by the water's content of carbonate, bicarbonate, hydroxide, and on occasion borate,

silicate, and phosphate. It is expressed in milligrams per liter of equivalent calcium carbonate (mg/L $CaCO_3$).

Analysis (chemical): the determination of the qualitative and/or quantitative composition of a substance.

Analyte: the substance, a property of which is to be measured by chemical analysis.

Analytical batch: a group of samples, including quality control samples, which are processed together using the same method, the same lots of reagents, and at the same time or in continuous, sequential time periods. Samples in each batch should be of similar composition and share common internal quality control standards.

Analytical reagent (AR): the American Chemical Society's designation for the highest purity of certain chemical reagents and solvents.

Anhydrous: a term meaning without water.

Background level (environmental): the concentration of substance in a defined control area during a fixed period of time before, during or after a data gathering operation.

Base: any substance which contains hydroxyl (OH) groups and furnishes hydroxide ions in solution; a molecular or ionic substance capable of combining with a proton to form a new substance; a substance that provides a pair of electrons for a covalent bond with an acid; a solution with a pH of greater than 7.

Batch: a quantity of material produced or processed in one operation, considered to be a uniform discrete unit.

Blank sample: a clean sample or a sample of matrix processed so as to measure artifacts in the measurement (sampling and analysis) process.

Calibrate: to determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the calibration standards should bracket the range of planned measurements.

Calibration curve: the graphical relationship between the known values for a series of calibration standards and instrument responses.

Calibration standard: a substance or reference material used to calibrate an instrument.

Calibration: the checking, adjusting, or systematic standardizing of the graduations of a quantitative measuring instrument.

Candidate method: a body of procedures and techniques of sample collection and/or analysis that is submitted for approval as a reference method, an equivalent method, or an alternative method.

Caustic soda: sodium hydroxide, NaOH.
Caustic: capable of destroying or eating away by chemical action; a hydroxide of a light metal.

Check sample: an uncontaminated sample matrix spiked with known amounts of analytes usually from the same source as the calibration standards. It is generally used to establish the stability of the analytical system but may also be used to assess the performance of all or a portion of the measurement system.

Check standard: a substance or reference material obtained from a source independent from the source of the calibration standard; used to prepare check samples.

Chemical analysis: the use of a standard chemical analytical procedures to determine the concentration of a specific analyte in a sample, or qualitatively or quantitatively measure a specific parameter of a sample.

Clean sample: a sample of a natural or synthetic matrix containing no detectable amount of the analyte of interest and no interfering material.

Colorimeters: A colorimeter is designed to perform a type of psychophysical sample analysis by mimicking human eye-brain perception, or designed to see color the way we do. If desired, this data may be compared to a standard or reference to determine acceptability. Colorimeters are accurate for straightforward color measurement and ideally suited for determination of color difference, fastness, and strength as well as routine comparisons of similar colors. They can be invaluable for color quality control and are primarily used in the production and inspection phases of manufacturing. While colorimeters can produce highly accurate color measurements, they also have several shortcomings; they are not able to identify metamerism or colorant strength, are not ideally suited for color formulation, and cannot be used under variable illuminant/observer conditions.

Composite sample: a sample prepared by physically combining two or more samples having some specific relationship and processed to ensure homogeneity.

Concentration: in solutions, the mass, volume, or number of moles of solute present in proportion to the amount of solvent or total solution Common measures are: molarity, normality, percent, molality, and by specific gravity scales.

Contamination: a general term signifying the introduction into water of microorganisms, chemicals, wastes or sewage which renders the water unfit for its intended use.

Data: facts or figures from which conclusions can be inferred.

Detection limit (DL): the lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated level of probability.

Determination: the application of the complete analytical process of measuring the property of interest in a sample, from selecting or measuring a test portion to the reporting of results.

Diluent: a substance added to another to reduce the concentration and resulting in a homogeneous end product without chemically altering the compound of interest.

Dilution factor: the numerical value obtained from dividing the new volume of a diluted substance by its original volume.

Dissolved protein: Protein substances that can dissolve in water.

Distilled water: water that has been purified by distillation (boiling the water off as steam and condensing it back to a liquid, leaving the impurities behind). Having been boiled, it is also sterile.

Document control: a systematic procedure for indexing documents by number, date and revision number for archiving, storage, and retrieval.

Duplicate analyses or measurements: the analyses or measurements of the

variable of interest performed identically on two subsamples of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory.

Duplicate samples: two samples taken from and representative of the same population and carried through all steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variance of the total method including sampling and analysis.

Duplicate: an adjective describing the taking of a second sample or performance of a second measurement or determination. Often incorrectly used as a noun and substituted for "duplicate sample." Replicate is to be used if there are more than two items.

Environmental sample: a sample of any material that is collected from an environmental source.

Environmentally related measurement: any assessment of environmental concern generated through or for field, laboratory, or modelling processes; the value obtained from such an assessment.

Equivalent method: any method of sampling and/or analysis demonstrated to result in data having a consistent and quantitatively known relationship to the results obtained with a reference method under specified conditions, and formally recognized by the USEPA.

Error (measurement): the difference between an observed or corrected value of a variable and a specified, theoretically correct, or true value.

External quality control: the activities which are routinely initiated and performed by persons outside of normal operations to assess the capability and performance of a measurement process.

Field blank: a clean sample (e.g., distilled water), carried to the sampling site, exposed to sampling conditions (e.g., bottle caps removed, preservatives added) and returned to the laboratory and treated as an environmental sample. Field blanks are used to check for analytical artifacts and/or background introduces by sampling and analytical procedures

Filtration: the process of separating solids from a liquid by means of a porous substance through which only the liquid can pass.

Good laboratory practices (GLP): either general guidelines or formal regulations for performing basic laboratory operations or activities that are known or believed to influence the quality and integrity of the results.

Grab sample: a single sample which is collected at one point in time and place.

Hazardous wastes: a hazardous waste is a material that is subject to special consideration by the USEPA, under 40CFR261. State or local authorities may also designate additional materials as hazardous waste in their areas. The definition given by 40 CFR 261 defines a hazardous waste as a solid waste that is not excluded from regulation and meets one or more of the following criteria: (a) it is a discarded commercial chemical product, off-specification species, container residue, or spill residue of materials specifically listed in 40CFR261.33 (P- and U-codes); (b) it is a waste from a specific source listed in 40CFR261.32 (K-code); (c) it is a waste from a non-specific source listed in 40CFR261.31 (F-code); and/or (d) it displays any of the following characteristics of hazardous wastes: ignitability

(such as flash point is below 60 degree C or 140 degree F, it it is classified by DOT as an oxidizer D001), corrosivity (such as the pH of the waste material is less than or equal to 2, or greater than or equal to 12.5, or classified by DOT as D002), reactivity (such as the waste material is unstable, reacts violently with water, may generate toxic gases when mixed with water, or classified by DOT as D003), or toxicity (such as it is classified by DOT ad D004-D043).

Instrument blank: a clean sample processed through the instrumental steps of the measurement process; used to determine instrument contamination. See Dynamic blank.

Interference: a positive or negative effect on a measurement caused by a variable other than the one being investigated.

Kjeldahl method: The Kjeldahl method was developed in 1883 by a brewer called Johann Kjeldahl. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. The Kjeldahl method does not measure the protein content directly a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration.

Management systems review (MSR): the qualitative assessment of a data collection operation and/or organization(s) to establish whether the prevailing

quality management structure, practices, and procedures are adequate for ensuring that the type and quality of data needed and expected are obtained.

Matrix: a specific type of medium (e.g., surface water, drinking water) in which the analyte of interest may be contained.

Measurement range: the range over which the precision and/or recovery of a measurement method are regarded as acceptable.

Measurement standard: a standard added to the prepared test portion of a sample (e.g. to the concentrated extract or the digestate) as a reference for calibrating and controlling measurement or instrumental precision and bias.

Median: the middle value for an ordered set of n values; represented by the central value when n is odd or by the mean of the two most central values when n is even.

Medium: a substance (e.g., air, water, soil) which serves as a carrier of the analytes of interest.

Method: a body of procedures and techniques for performing a task (e.g., sampling, characterization, quantification) systematically presented in the order in which they are to be executed.

Optical density: it is measured in a spectrophotometer, can be used as a measure of the concentration of substances in a suspension. As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria or other material is present. Optical density measures the amount of attenuation, or intensity lost, when light passes through an optical component. It also tracks attenuation based on the scattering of light, whereas absorbance considers only the absorption of light within the optical component.

Parameter: any quantity such as a mean or a standard deviation characterizing a population. Commonly misused for "variable", "characteristic" or "property."

pH adjustment: a means of maintaining the optimum pH through the use of chemical additives.

pH: the negative logarithm of the hydrogen ion concentration ($-\log10[H^+]$) where H^+ is the hydrogen-ion concentration in moles per liter. Neutral water has a pH value of 7.

Precision: the degree to which a set of observations or measurements of the same property, usually obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms.

Procedure: a set of systematic instructions for performing an operation.

Protein: Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.*, tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique functional properties, *i.e.*, their ability to provide desirable appearance,

texture or stability.

Quality assurance (QA): an integrated system of activities involving planning, quality control, quality assessment, reporting and quality improvement to ensure that a product or service meets defined standards of quality with a stated level of confidence.

Quality control (QC): the overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users. The aim is to provide quality that is satisfactory, adequate, dependable, and economical.

Quality: the sum of features and properties/characteristics of a product or service that bear on its ability to satisfy stated needs.

Random: lacking a definite plan, purpose or pattern; due to chance.

Range: the difference between the minimum and the maximum of a set of values.

Raw data: any original factual information from a measurement activity or study recorded in laboratory worksheets, records, memoranda, notes, or exact copies thereof and that are necessary for the reconstruction and evaluation of the report of the activity or study. Raw data may include photographs, microfilm or microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments. If exact copies of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted.

Reagent blank: a sample consisting of reagent(s), without the target analyte or sample matrix, introduced into the analytical procedure at the appropriate point and

carried through all subsequent steps to determine the contribution of the reagents and of the involved analytical steps to error in the observed value.

Reagent grade: the second highest purity designation for reagents which conform to the current specifications of the American Chemical Society Committee on Analytical Reagents.

Reagent: a chemical substance used to cause a reaction for the purpose of chemical analysis.

Reduction treatment: the opposite of oxidation treatment wherein a reductant is used to lower the valence state of a pollutant to a less toxic form; e.g. the use of SO_2 to reduce Cr^{6+} to Cr^{3+} in an acidic solution.

Reduction: chemical reaction in which an atom or molecule gains an electron; decrease in positive valence; addition of hydrogen to a molecule.

Sample variance (statistical): a measure of the dispersion of a set of values. The sum of the squares of the difference between the individual values of a set and the arithmetic mean of the set, divided by one less than the number of values in the set. (The square of the sample standard deviation.)

Sample: a part of a larger whole or a single item of a group; a finite part or subset of a statistical population. A sample serves to provide data or information concerning the properties of the whole group or population.

Sampling: the process of obtaining a representative portion of the material of concern.

Solution: a liquid (solvent) that contains a dissolved substance (solute).

Solvent: a liquid used to dissolve another substance.

Spectrophotometers: A spectrophotometer is an instrument designed for physical sample analysis via full spectrum color measurement. By providing wavelength-by-wavelength spectral analysis of a sample's reflectance, absorbance, or transmittance properties, it produces precise data beyond that observable by the human eye. Spectrophotometers offer a higher level of flexibility and versatility than colorimeters due in part to the fact that they offer multiple illuminant/observer combinations and are capable of measuring metamerism, identifying colorant strength, analyzing a comprehensive range of sample types, and giving users a choice between including or excluding specular reflectance to account for geometric attributes. Full spectrum analysis also provides for greater specificity, potentially identifying color differences missed by colorimeters.

Spiked reagent blank: a specified amount of reagent blank fortified with a known mass of the target analyte; usually used to determine the recovery efficiency of the method.

Spiked sample: a sample prepared by adding a known mass of target analyte to a specified amount of matrix sample for which an independent estimate of target analyte concentration is available. Spiked samples are used, for example, to determine the effect of the matrix on a method's recovery efficiency.

Split samples: two or more representative portions taken from a sample or subsample and analyzed by different analysts or laboratories. Split samples are used to replicate the measurement of the variable(s) of interest.

Standard (measurement): a substance or material with a property quantified with sufficient accuracy to permit its use to evaluate the same property in a similar substance or material. Standards are generally prepared by placing a reference

material in a matrix.

Standard addition: the procedure of adding known increments of the analyte of interest to a sample to cause increases in detection response. The level of the analyte of interest present in the original sample is subsequently established by extrapolation of the plotted responses.

Standard deviation: the most common measure of the dispersion or imprecision of observed values expressed as the positive square root of the variance.

Standard method: an assemblage of techniques and procedures based on consensus or other criteria, often evaluated for its reliability by collaborative testing and receiving organizational approval.

Standard operating procedure (SOP): a written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

Standard reference material (SRM): a certified reference material produced by the U.S. National Institute of Standards and Technology and characterized for absolute content independent of analytical method.

Standard solution: a solution containing a known concentration of analytes, prepared and verified by a prescribed method or procedure and used routinely in an analytical method.

UV-visible spectroscopy: A number of methods have been devised to measure protein concentration, which are based on UV-visible spectroscopy. These methods use either the natural ability of proteins to absorb (or scatter) light in the UV-

visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb (or scatter) light in this region. The basic principle behind each of these tests is similar. First of all a calibration curve of absorbance (or turbidity) versus protein concentration is prepared using a series of protein solutions of known concentration. The absorbance (or turbidity) of the solution being analyzed is then measured at the same wavelength, and its protein concentration determined from the calibration curve. The main difference between the tests are the chemical groups which are responsible for the absorption or scattering of radiation, *e.g.*, peptide bonds, aromatic side-groups, basic groups and aggregated proteins.

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EDITORS PAGE

Editors of

"EVOLUTIONARY PROGRESS IN SCIENCE, TECHNOLOGY, ENGINEERING, ARTS AND MATHEMATICS (STEAM)"

1. Dr. Lawrence K. Wang (王抗曝)

Lawrence K. Wang has over 30+ years of professional experience in facility design, environmental sustainability, natural resources, STEAM education, global pollution control, construction, plant operation, and management. He has expertise in water supply, air pollution control, solid waste disposal, drinking water treatment, waste treatment, and hazardous waste management. He was the Director/Acting President of the Lenox Institute of Water Technology, Engineering Director of Krofta Engineering Corporation and Zorex Corporation, and a Professor of RPI/SIT/UIUC, in the USA. He was also a Senior Advisor of the United Nations Industrial and Development Organization (UNIDO) in Austria. Dr. Wang is the author of over 700 technical papers and 45+ books, and is credited with 24 US patents and 5 foreign patents. He earned his two HS diplomas from the High School of National Taiwan Normal University and the State University of New York. He also earned his BS degree from National Cheng-Kung University, Taiwan, ROC, his two MS degrees from the University of Missouri and the University of Rhode Island, USA, and his PhD degree from Rutgers University, USA.

Currently he is the Chief Series Editor of the Handbook of Environmental Engineering series (Springer); Chief Series Editor of the Advances in Industrial and Hazardous Wastes Treatment series, (CRC Press, Taylor & Francis); co-author of the Water and Wastewater Engineering series (John Wiley & Sons); Co-Series Editor of the Handbook of Environment and Waste Management series (World Scientific) and Co-Series Editor of the Evolutionary Progress in Science, Technology, Engineering, Arts and Mathematics (Lenox Press). Dr. Wang is active in professional activities of AWWA, WEF, NEWWA, NEWEA, AIChE, ACS, OCEESA, etc.

2. Dr. Hung-ping Tsao (曹恆平)

Hung-ping Tsao has been a mathematician, a university professor, and an assistant actuary, serving private firms and universities in the United States and Taiwan for 30+ years. He used to be an Associate Member of the Society of Actuaries and a Member of the American Mathematical Society. His research have been in the areas of college mathematics, actuarial mathematics, management mathematics, classic number theory and Sudoku puzzle solving.

In particular, bikini and open top problems are presented to share some intuitive insights and some type of optimization problems can be solved more efficiently and categorically by using the idea of the boundary being the marginal change of a well-rounded region with respect to its inradius; theory of interest, life contingency functions and pension funding are presented in more simplified and generalized fashions; the new way of the simplex method using cross-multiplication substantially simplified the process of finding the solutions of optimization problems; the generalization of triangular arrays of numbers from the natural sequence based to arithmetically progressive sequences based opens up the dimension of explorations; the introduction of step-by-step attempts to solve Sudoku puzzles makes everybody's life so much easier and other STEAM project development.

Dr. Tsao is the author of 3 books and over 30 academic publications. He earned his high school diploma from the High School of National Taiwan Normal University, his BS and MS degrees from National Taiwan Normal University, Taipei, Taiwan, his second MS degree from the UWM in USA, and a PhD degree from the University of Illinois, USA. Currently Dr. Tsao is the Co-Series Editor of the "Evolutionary Progress in Science, Technology, Engineering, Arts and Mathematics" eBook series (Lenox Press).

Editors of the eBOOK Series of the *"EVOLUTIONARY PROGRESS IN SCIENCE, TECHNOLOGY, ENGINEERING, ARTS AND MATHEMATICS (STEAM)"*

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Dr. Hung-ping Tsao (曹恆平) -- right

E-BOOK SERIES AND CHAPTER INTRODUCTON

Introduction to the eBOOK Series of :

the *"EVOLUTIONARY PROGRESS IN SCIENCE,*

TECHNOLOGY, ENGINEERING, ARTS AND MATHEMATICS (STEAM)"

and This Chapter

"A SPECTROPHOTOMETRICAL METHOD FOR

DETERMINATION OF DISSOLVED PROTEINS

IN WATER OR WASTEWATER"

The acronym STEM stands for "science, technology, engineering and mathematics". In accordance with the National Science Teachers Association (NSTA), "A common definition of STEM education is an interdisciplinary approach to learning where rigorous academic concepts are coupled with real-world lessons as students apply science, technology, engineering, and mathematics in contexts that make connections between school, community, work, and the global enterprise enabling the development of STEM literacy and with it the ability to compete in the new economy". The problem of this country has been pointed out by the US Department of Education that "All young people should be prepared to think deeply and to think well so that they have the chance to become the innovators, educators, researchers, and leaders who can solve the most pressing challenges facing our nation and our world, both today and tomorrow. But, right now, not enough of our youth have access to quality STEM learning opportunities and too few students see these disciplines as springboards for their careers."

STEM learning and applications are very popular topics at present, and STEM related careers are in great demand. According to the US Department of Education reports that the number of STEM jobs in the United States will grow by 14% from 2010 to 2020, which is much faster than the national average of 5-8 % across all job sectors. Computer programming and IT jobs top the list of the hardest to fill jobs. Despite this, the most popular college majors are business,law, etc., not STEM related. For this reason, the US government has just extended a provision allowing foreign students that are earning degrees in STEM fields a seven month visa extension, now allowing them to stay for up to three years of "on the job training". So, at present STEM is a

legal term. The acronym STEAM stands for “science, technology, engineering, arts and mathematics”. As one can see, STEAM (adds “arts”) is simply a variation of STEM.

The word of “arts” means application, creation, ingenuity, and integration, for enhancing STEM inside, or exploring of STEM outside. It may also mean that the word of “arts” connects all of the humanities through an idea that a person is looking for a solution to a very specific problem which comes out of the original inquiry process. The acronym STEAM stands for “science, technology, engineering arts and mathematics”. STEAM is an academic new term in the field of education.

The University of San Diego and Concordia University offer a college degree with a STEAM focus. Basically STEAM is a framework for teaching or R&D, which is customizable and functional, thence the “fun” in functional. As a typical example, if STEM represents a normal cell phone communication tower looking like a steel truss or concrete column, STEAM will be an artificial green tree with all devices hided, but still with all cell phone communication functions. This ebook series presents the recent evolutionary progress in STEAM with many innovative chapters contributed by academic and professional experts.

This ebook chapter, *“A SPECTROPHOTOMETRICAL METHOD FOR DETERMINATION OF DISSOLVED PROTEINS IN WATER OR WASTEWATER"* is the authors’ collection of thoughts, chemical experimental works and literature articles about feasible ways of analyzing dissolved protein in a complex aquatic environment of water and wastewater. Specifically the authors initially review current methods for rapid analysis of dissolved protein in water and wastewater, and subsequently introduce a new spectrophotometrical method for determination of dissolved proteins in the 10-2500 mg/L range using carbonate-tartrate reagent, phosphomolybdic-phosphotungstic reagent, and copper sulfate. The new analytical method is a colorimetric determination based on the reaction of dissolved protein, specifically the peptide bonds and the amino acids tryptophan and tyrosine with the authors suggested reagents. Two reactions are necessary:(a) The reaction between the protein molecules and copper ions in an alkaline solution; and (b) The reduction of a phosphomolybdic phototungstic reagent by the copper treated protein. The optical density of treated dissolved protein is then determined at 400-700 nm range using a

spectrophotometer with 1 cm light path or longer. With certain modification, this method may also be used in the field using the calibration curves prepared in the laboratory.